Citation:

de Jong AE, Verhoeff-Bakkenes L, Nauta MJ, de Jonge R. Cross-contamination in the kitchen: Effect of hygiene measures. *J Appl Microbiol*; 105 (2): 615-624 2008 Aug;105 (2): 615-624. Epub 2008 Mar 12.

PubMed ID: 18341559

Study Design:

Laboratory simulation study

Class:

C - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine the effect of hygiene measures to prevent the transfer of *C. jejuni* from chicken meat to a prepared meal, due to cross-contamination via hands, cutlery and cutting boards.

Inclusion Criteria:

Not applicable; cellular study.

Exclusion Criteria:

Not applicable; cellular study.

Description of Study Protocol:

Recruitment

Not applicable; cellular study.

Design

- Comparative tests were conducted with non-pathogenic *Escherichia coli* (gram negative), *Lactobacillus casei* (gram positive) and *L. casei* was chosen as the safe tracer organism. In order to select an appropriate tracer organism for *C. jejuni*, comparative tests were conducted with meat and salad storage trials, meat treatment trials and salad preparation trials
- The study was conducted in a private kitchen using regularly used cutlery and cutting boards instead of standardized laboratory tools. The inoculated fillets were stored in similar conditions to that of typical supermarkets and households. The food handling practices employed were based on consumer-style methods rather than international food safety

guidelines
- Most Probable Number (MPN) method and spread-plating suitable dilutions on agar plates determined contamination levels of fillets and salad
- Chicken breasts were inoculated with a known contamination level of bacteria and salad contamination levels were measured
- The selected recipe allowed for access to all important cross-contamination contact points (hands, cutting boards and cutlery); offered a standardized cooking method (boiling) to allow set preparation times and temperature
- Chicken and salad were homogenated via a blender. Each was further diluted in peptone physiological salt solution
- Preparation of inocula and strain cocktails: Experiments were conducted with either single- or multiple-species cultures (*C. Jejuni*: Five-strain cocktail used (strains mixed in equal volumes) as single species inoculums; *L. casei*: Adjusted to pH 6.8-7.2 with NaOH before use to neutralize pH effects with combining with other organisms; multiple species cultures were prepared by mixing equal volumes of single-species inocula)
- Meat Inoculation: Chicken breast fillets (100-150g) were bought in different batches a local grocery store; used fresh or were stored frozen and defrosted before use. Inoculation levels ranged between 108 and 109 CFU per fillet. Each contaminated fillet stored overnight in a separate plastic bag at 4°C to imitate retail storage conditions and to allow bacterial cells to attach to the meat
- Storage trials and comparative tests: All experiments were conducted in duplicate with both single and multiple-species inoculation cultures. Comparative tests results was used to help select the tracer organisms.
- Meat Storage Trial: Assessed if overnight storage affected the recovery of the bacteria used; cell counts conducted at zero, one, three, five and 24 hours; the effect of active manually washing fillets [cold (10°C) running water for 10 seconds] and passive cleaning [soaking fillets in cold chicken stock made of stock cubes for 10 minutes] of cold-stored fillets on the recovery of inoculated bacteria was determined in time (one and 24 hours)
- Salad Storage Trial: Assessed if overnight storage affected the recovery of the bacteria used; cells counts of cold-stored salads were measured at zero, three, five and 24 hours.

Dietary Intake/Dietary Assessment Methodology

Not applicable; cellular study.

Blinding Used

Not applicable; cellular study.

Intervention

- Salads containing chicken breast fillet contaminated with a known number of *C. jejuni* and *L. casei* were prepared according to different cross-contamination scenarios and contamination levels of salads were determined
- The intervention or treatment for this study included applying different cross-contamination routes
- Only the effect of different washing protocols to reduce cross-contamination via hands (by direct contact only), cutlery and cutting boards were examined.

Statistical Analysis

- No statistical analyses were conducted
- A formula was utilized to express the number of cells present in the salad, because the cell

counts of inoculation cultures differed for each species. Cell numbers given are indicative for the number of cells recoverable from a salad if the chicken meat would have been contaminated with 109 CFU.

- Formula: $\log N_{salad} - \log N_{chicken} + 9$.

Data Collection Summary:

Timing of Measurements

- Meat Storage Trial: Cell counts conducted at zero, one, three, five and 24 hours. Washing impact on cold-stored fillets on the recovery of inoculated bacteria was determined at one and 24 hours
- Salad Storage Trial: Cells counts of cold-stored salads were measured at zero, three, five and 24 hours
- Salads: Analyzed on the same day or after overnight storage.

Dependent Variables

Cell counts of *C. jejuni* and *L. casei* in the salad.

Independent Variables

Cross-contamination routes: Hands, cutlery and cutting boards.

Control Variables

Amount and type of bacteria inoculated in each fillet.

Description of Actual Data Sample:

- *Initial N:* Not applicable; cellular study
- *Attrition (final N):* Not applicable; cellular study
- *Age:* Not applicable; cellular study
- *Ethnicity:* Not applicable; cellular study
- *Other relevant demographics:* Not applicable; cellular study
- *Anthropometrics:* Not applicable; cellular study
- *Location:* The Netherlands.

Summary of Results:

Key Findings

- High contamination levels of both micro-organisms were observed in salads when cross-contamination via cutting board, cutlery or hands was not prevented
- Cross-contamination of *C. jejuni* via cutting board was strongly decreased to nearly undetectable levels when the cutting board was rinsed for 10 seconds under hot water
- Washing cutting boards with hot water and detergent resulted in higher contamination levels of the salads than only using hot water as a rinse; using a cold water rinse hardly affected cell counts compared with unwashed cutting boards
- Rinsing cutlery with hot water or washing with hot water and soap resulted in undetectable

cell levels in the salads for *C. jejuni*, while this effect was only partly achieved when cutlery was washed using hot water and soap for *L. casei*
- Cross-contamination of *C. jejuni* via hands was decreased when using cold water and soap when washing hands; rinsing with cold water alone was somewhat less effective
- *L. casei* was poorly removed when rinsing with cold water alone.

Other Findings

- The meat storage trial showed contamination levels of fillets were stable over time (zero hours vs. 24 hours) for all organisms tested, both in pure as in mixed culture although there was some loss in cell counts was seen between the number of cells applied to fillets and those recovered from the fillets following inoculation (T=0 hours)
- The salad storage trial showed that for pure cultures cell counts remained stable in refrigerated salads; cell counts varied only by 0.1 log N per salad over time. For mixed cultures this range increased to 0.3 log N per salad for *E. coli* and to 0.5 log N per salad for *C. jejuni*
- The meat treatment trial showed some deviation in test results of *E. coli* and the other organisms tested
- At all time points, actively cleaning the fillets decreased cell counts by one log cycle except for *E. coli* after one hour storage. The results were similar for pure and mixed cultures.
- Salad preparation trials revealed that *C. jejuni* and both target organisms behaved comparably when salads were prepared according to best-case and worst-case scenarios
- Comparative tests indicated that fillets and salads could be stored overnight without influencing the recovery rates
- Using mixed cultures barely affected cell counts of individual species
- Both trace organisms were similar to *C. jejuni*, indicating that *E. coli* and *L. casei* can be considered suitable tracer organisms.

Author Conclusion:

- Dishwashing does not sufficiently prevent cross-contamination, thus different cutting boards for raw meat and other ingredients should be used
- Meat-hand contact should be avoided or hands should be thoroughly cleaned with soap
- *L. casei* can be used as a safe tracer organism for *C. jejuni* in consumer observation studies.

Reviewer Comments:

- *Limitation: The authors did not state who prepared the food; it is unknown if volunteers or trained researchers prepared the food. If the researchers did so, although they tried to mimic "real life" scenarios, they may have unintentionally utilized better practices than the average consumer*
- *Study strengths: The recipe used standardized temperatures and took into account cooking preferences of consumers (e.g. consumers may or may not wash meat prior to use), utilized consumer practices vs. international recommendations*
- *Authors noted this limitation: The data alone do not allow drawing conclusion on the importance of each hygiene measure*
- *Authors noted these strengths: Experiments were performed in a private kitchen using regular cutting boards and cutlery rather than laboratory tools; and food handling and*

handwashing practices followed standard consumer practices rather than recommended practices.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	???

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	N/A
2.	**Was the selection of study subjects/patients free from bias?**	N/A
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	N/A
2.2.	Were criteria applied equally to all study groups?	N/A
2.3.	Were health, demographics, and other characteristics of subjects described?	N/A
2.4.	Were the subjects/patients a representative sample of the relevant population?	N/A
3.	**Were study groups comparable?**	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A

3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	N/A
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	N/A
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	N/A
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	N/A
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes

6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	Yes
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	N/A
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	???
7.5.	Was the measurement of effect at an appropriate level of precision?	???
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	???
8.1.	Were statistical analyses adequately described and the results reported appropriately?	N/A
8.2.	Were correct statistical tests used and assumptions of test not violated?	N/A
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	N/A
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A

8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	N/A
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes